



Negative Selection  
Catalog #19758

## EasySep™ Mouse Epithelial Cell Enrichment Kit

For processing  $1 \times 10^9$  cells



Scientists Helping Scientists™ | [WWW.STEMCELL.COM](http://WWW.STEMCELL.COM)

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713  
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM  
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Document #29165 | Version 1\_1\_2

## Description

Enrich untouched and highly purified epithelial cells from freshly dissociated mouse mammary tissues by immunomagnetic negative selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast, easy-to-use and column-free
- Isolated cells are untouched

This kit targets non-epithelial cells for removal with biotinylated antibodies recognizing non-epithelial cell surface markers. Unwanted cells are labeled with biotinylated antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

## Component Descriptions

| COMPONENT NAME                                     | COMPONENT # | QUANTITY   | STORAGE                             | SHELF LIFE                               | FORMAT  |
|--|-------------|------------|-------------------------------------|--|---|
| EasySep™ Mouse Epithelial Cell Enrichment Cocktail | 19757C.1    | 1 x 0.5 mL | Store at 2 - 8°C.<br>Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS. Includes an Fc receptor blocking antibody. |
| EasySep™ Mouse Biotin Selection Cocktail           | 19153       | 1 x 1 mL   | Store at 2 - 8°C.<br>Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS.  |
| EasySep™ Magnetic Nanoparticles                    | 19150       | 1 x 1 mL   | Store at 2 - 8°C.<br>Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles in water.  |

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

## Sample Preparation

### DISSOCIATION OF MOUSE MAMMARY TISSUE

Use Gentle Collagenase/Hyaluronidase (Catalog #07919) to enzymatically digest mouse mammary tissue. Refer to the associated Product Information Sheet (Document #29629) for detailed information on the recommended protocol. For more information, visit [www.stemcell.com](http://www.stemcell.com) or contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com)

After preparation, resuspend cells at  $1 \times 10^8$  cells/mL in recommended medium.



## Recommended Medium



HBSS with 10 mM HEPES, Without Phenol Red (Catalog #37150) containing 2% fetal bovine serum (FBS).

## Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

**Table 1. EasySep™ Mouse Epithelial Cell Enrichment Kit Protocol**

|      |  | EASYSEP™ MAGNETS  |  |
|------|--|---|--|
| STEP | INSTRUCTIONS   |  <b>EasySep™</b><br>(Catalog #18000)                 |  <b>“The Big Easy”</b><br>(Catalog #18001)          |
| 1    | Prepare sample at the indicated cell concentration within the volume range.  | 1 x 10 <sup>8</sup> cells/mL<br>0.2 - 2 mL<br>NOTE: If starting with fewer than 2 x 10 <sup>7</sup> cells, resuspend cells in 0.2 mL. | 1 x 10 <sup>8</sup> cells/mL<br>0.5 - 8 mL   |
| 2    | Add DNase to sample.   | 100 µg/mL of sample   | 100 µg/mL of sample  |
| 3    | Add sample to required tube.   | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)   | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Corning Catalog #38008)   |
| 4    | Add Enrichment Cocktail to sample.   | 50 µL/mL of sample  | 50 µL/mL of sample   |
|      | Mix and incubate.  | 2 - 8°C for 15 minutes  | 2 - 8°C for 15 minutes   |
| 5    | Add Selection Cocktail to sample.  | 100 µL/mL of sample   | 100 µL/mL of sample  |
|      | Mix and incubate.  | 2 - 8°C for 15 minutes  | 2 - 8°C for 15 minutes   |
| 6    | Mix Magnetic Particles.<br>NOTE: Particles should appear evenly dispersed.   | Pipette up and down more than 5 times   | Pipette up and down more than 5 times  |
| 7    | Add Magnetic Particles to sample.  | 50 µL/mL of sample  | 50 µL/mL of sample   |
|      | Mix and incubate.  | 2 - 8°C for 15 minutes  | 2 - 8°C for 15 minutes   |
| 8    | Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.                | Top up to 2.5 mL  | <ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 1 mL</li> <li>• Top up to 10 mL for samples ≥ 1 mL</li> </ul> |
|      | Place the tube (without lid) into the magnet and incubate.   | RT for 5 minutes  | RT for 5 minutes   |
| 9    | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.  | Use a new 5 mL tube   | Use a new 14 mL tube   |
| 10   | Remove the tube from the magnet and add recommended medium to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. | Add 2 mL  | Add 2 mL   |
|      | Replace the tube (without lid) into the magnet and incubate.   | RT for 5 minutes  | RT for 5 minutes   |
| 11   | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension.                  | Combine with poured-off fraction from step 9  | Combine with poured-off fraction from step 9   |

|      |   | EASYSEP™ MAGNETS  |  |
|------|---|---|--|
| STEP | INSTRUCTIONS (CONTINUED)  |  <b>EasySep™</b><br>(Catalog #18000) | <b>"The Big Easy"</b><br>(Catalog #18001)           |
| 12   | Centrifuge the enriched cell suspension.  | 300 x g for 5 minutes   | 300 x g for 5 minutes  |
| 13   | Discard the supernatant and resuspend the cell pellet in recommended medium to the indicated volume.                                | Top up to 2.5 mL  | <ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 1 mL</li> <li>• Top up to 10 mL for samples ≥ 1 mL</li> </ul> |
|      | Place the tube (without lid) into the magnet and incubate.  | RT for 5 minutes  | RT for 5 minutes   |
| 14   | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube. | Use new 5 mL tube   | Use new 14 mL tube   |
| 15   | Centrifuge the enriched cell suspension.  | 300 x g for 5 minutes   | 300 x g for 5 minutes  |
| 16   | Discard supernatant and resuspend cells in desired medium.  | Isolated cells are ready for use  | Isolated cells are ready for use   |

RT - room temperature (15 - 25°C)

\* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

## Notes and Tips

### ASSESSING PURITY

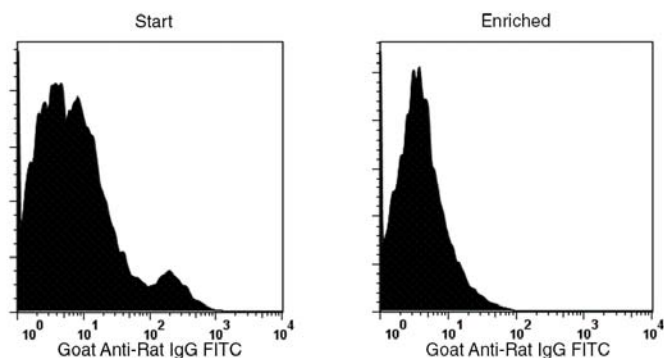
For purity assessment of epithelial cells by flow cytometry use the following fluorochrome-conjugated antibody:

- FITC goat anti-rat IgG (e.g. Jackson ImmunoResearch Catalog #112-095-167)

### SELECTION OF MOUSE MAMMARY STEM CELLS

For further selection of mouse mammary luminal, basal, and stem cells, incubate the epithelial-enriched cell preparation with antibodies specific to CD24 (e.g. Anti-Mouse CD24 Antibody, Clone M1/69, PE; Catalog #60099PE) and CD49f (e.g. Anti-Mouse CD49f Antibody, Clone GoH3, FITC; Catalog #60037FI). EpiCult™- B Mouse Medium Kit (Catalog #05610) can be used as a medium for growth and culture of mouse mammary progenitor cells.

## Data



In the above example, the percentage of hematopoietic, endothelial, and fibroblast cells in the start and enriched fractions are  $10.4 \pm 0.5\%$  and  $1.1 \pm 0.2\%$ , respectively.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485. PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

Copyright © 2017 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, and EasySep are trademarks of STEMCELL Technologies Canada Inc. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.